Universidade de Aveiro Departamento de Biologia

Ano 2016

Daniela Maria Justino Alves Ramos

Comportamento agonístico intraespecífico durante eventos consecutivos de assentamento de um invertebrado marinho com um ciclo de vida bifásico

Intraspecific agonistic behaviour during settlement events of a marine invertebrate with a biphasic life cycle

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Dissertação apresentação à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica do Professor Doutor Ricardo Calado, do Departamento de Biologia da Universidade de Aveiro.

o júri

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Agradecimentos: Ao meu orientador Ricardo Calado por me ter guiado e exigido o melhor ao longo deste trabalho e à Gina Neto por me ter acompanhado e ajudado em todos os trabalhos de campo, laboratoriais e escrita.

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E por fim à minha família, por todo o apoio incondicional e inspiração.

Palavras-chave *Carcinus maenas*, megalopa, juvenis, canibalismo, decápode, comportamento.

Resumo O canibalismo é um comportamento intraespecífico observado entre muitas espécies de invertebrados marinhos, introduzindo alterações na dinâmica e distribuição das populações. Neste estudo pretendemos testar se este comportamento pode ser observado durante o assentamento e pós-assentamento de um decápode com um ciclo de vida complexo. Megalopas selvagens do caranguejo braquiúro *Carcinus maenas* foram recolhidas na Ria de Aveiro (Portugal) e em condições de laboratório, testou-se a sua sobrevivência quando expostas a diferentes densidades, complexidade de habitat e disponibilidade de alimento. Foi também testada a ocorrência de canibalismo dos três primeiros estágios de juvenis para com conspecíficos no estado larvar de megalopa. Os resultados experimentais revelaram que o canibalismo não é comum entre megalopas, sendo apenas influenciado quando estão presentes em grandes densidades. Os juvenis no terceiro estágio de caranguejo (C3) apresentaram comportamentos canibalísticos imediatos e mais significativos quando comparados com os juvenis do primeiro e segundo estágio de caranguejo (C1 e C2). Os resultados apresentados permitiram deste modo perceber que durante elevados picos de assentamento, o desempenho e sobrevivência das megalopas não é afetado por possíveis comportamentos canibalísticos entre estas. No entanto, a estrutura das populações adultas pode não corresponder ao potencial de assentamento das larvas quando coespecíficos de eventos anteriores, como por exemplo, juvenis C3, se encontram presentes no mesmo local, devido à ocorrência de canibalismo.

Key-words *Carcinus maenas*, megalopae, juveniles, cannibalism, decapod, behaviour.

Abstract Cannibalism is a well-known behaviour among several invertebrate marine species and may play a relevant role in population dynamics and distribution. In this study we tested if this behaviour occurs at key moments of the complex life cycle of a decapod, namely at settlement and early post-metamorphosis. We collected wild megalopae of the brachyuran crab *Carcinus maenas* at Ria de Aveiro (Portugal) and at laboratory conditions tested the survival of megalopae stocked at different densities and under contrasting conditions of habitat and food availability. We also tested if cannibalism could be observed between megalopae and first, second and third instar juvenile crabs. Our results showed that megalopae only display cannibalism towards conspecifics at the same life stage under high densities scenarios. The occurrence of cannibalism towards megalopae was immediate and more significant with juveniles on the third crab stage (C3) when compared with the first and second juvenile crab stage (C1 and C2). This study allowed to perceived that during high settlement peaks, the performance and survival of megalopae is not affected by possible cannibalistic interactions among other megalopae. However, the structure and number of the adult population may not correspond to the potential settlement of the larvae when conspecifics of previous events, such as juvenile C3 are present in the same place, due to occurrence of cannibalism.

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1. Introduction

1.1. Invertebrates with biphasic life cycle

A wide group of marine invertebrates displays biphasic life cycles and presents distinct live forms throughout their development, e.g., as a free swimming larvae and benthic adults (Pechenik, 1998, 1999; Calado and Leal, 2015). The pre reproductive pelagic form develops in the water column until it becomes a competent larvae and is able to settle (Forward et al., 2001). Organisms like brachyuran crabs have a transition larval stage between the planktonic zoae and benthic crabs termed megalopa; this larval life stage displays an efficient swimming performance (Forward et al., 2001). Megalopae are able to move vertically, thus facilitating cues reception and enhancing the chances of finding a suitable habitat (Queiroga, 1998; Forward et al., 2001; Queiroga et al., 2006). Once megalopae are able to settle, they dwell in the plankton for hours, days or even weeks depending on the existence of certain chemical or physical environmental cues (Pechenik, 1998; Forward et al., 2001; Calado and Leal, 2015). Such cues can be present in different forms, such as the presence of other co-specifics, food availability, distinct substrata or water currents (Forward et al., 2001; Jackson, 2002; Queiroga et al., 2006). This specific stimulus acts as an activation for the megalopa to settle and become a functional benthic juvenile (Pechenik, 1998; Pechenik, 2006; Forward et al., 2001; Calado and Leal, 2015). The transition between the two life forms is called metamorphosis, linking the whole cycle; it is considered a very important and complex development step, as the organism faces dramatic changes at a morphological, physiological and behavioural level (Pechenik et al., 1998; Pechenik, 2006; Ventura et al., 2008; Calado and Leal, 2015).

Life adaptations like this are described as ancient and preserved in the evolutionary history of the marine environment (Pechenik, 1999; Jackson, 2002; Bishop and Brandhorst, 2003). As such, it displays a number of advantages and disadvantages (Vence, 1973; Pechenik et al., 1998). Planktotrophic larvae have the ability of selecting tidal streams as a transport mechanism by moving vertically in the water column (Yamada, 2001; Queiroga, 1998; Queiroga et al., 2002; Queiroga et al., 2006). As an outcome, when exposed to marine currents

they get dispersed from its original habitat facilitating the connectivity and colonization of new ones (Calado and Leal, 2015). Having different food resources and settling on a different environment prevents original habitat saturation and possible encounters with adult conspecifics. Dwelling among the plankton also decrease predation related to the benthos environment (Pechenik, 1999; Forward et al., 2001).

However, larvae are limited swimmers and staying in the water column for a longer period of time increases the probability of being predated and dispersed to a point where habitat may no longer be suitable for the species to settle (Vance, 1973; Pechenik et al, 1998). A mismatch or absence of a particular cue may cause a delay in metamorphosis in already competent larvae (Forward et al., 2001; Pechenik, 2006; Calado and Leal, 2015). Surpassing the first phase of the cycle, benthic juveniles become fully prepared to live and develop in the benthos. Once settled, young cohorts have to seek for suitable refuge habitats in order to avoid predation, the number one cause of juvenile mortality (Hines and Ruiz, 1995; Lovrich and Sainte-Marie, 1997; Luppi et al., 2001).

Cannibalism is also known to be a regular intraspecific behaviour among marine invertebrates with both pelagic and benthic forms. Previous studies have shown the occurrence of intraspecific predation in many invertebrate species, including crustaceans such as *Chionoecetes opilio* (Dutil et al., 1997), *Callinectes sapidus* (Ryer et al., 1997), *Portunus pelagicus* (Marshall et al., 2005), *Chasmagnathus granulata* and *Cyrtograpsus angulatus* (Luppi et al., 2001), *Paralithodes camtschaticus* (Stevens and Swiney, 2005), *Ucides cordatus* (Ventura et al., 2008; Ventura et al., 2011) and *Carcinus maenas* (Moksnes, 2004). Along the years, cannibalism was proven to be an important biological interaction mechanism that controls population dynamics and shapes their structure (Fox, 1975; Hedvall et al., 1998; Moksnes, 2004b). Some specific factors are believed to be the main influence for triggering this behaviour, such as refuge availability (Moksnes et al., 1997; Moksnes et al., 1998; Luppi et al., 2001; Marshall et al., 2005), habitat complexity (Ryer et al.,1997; Hedvall et al., 1998; Moksnes et al., 1998; Moksnes, 2002; Stevens and Swiney, 2005; Stoner et al., 2010), food sources (Lovrich and Sainte-Marie, 1997; Luppi et al., 2001), density (Lovrich and Sainte-Marie, 1997; Moksnes et al., 1997; Moksnes, 2002; Moksnes, 2004b) , body size (Dutil et al., 1997; Lovrich and Sainte-Marie, 1997; Luppi et al., 2001;

Marshall et al., 2005; Almeida et al., 2011) and moulting stages (Ryer et al.,1997; Marshall et al., 2005).

The present study addresses a number of these factors using the brachyuran crab *C. maenas*, a common species in estuarine ecosystems, as a model.

1.2. Ria de Aveiro

1.2.1. Characterization of study area

Estuaries are transitional and complex system between land and sea with continued exchanges of marine and fresh water masses (Klassen and Locke, 2007). These areas contain dynamic ecosystems with high productivity that create favourable habitats for several valuable species, such as marine birds, fish, and benthic invertebrates (Amaral et al., 2009; Lillebø et al., 2011).

Ria de Aveiro is a bar-built estuary located at northwest coast of mainland Portugal. It is semi-isolated from the Atlantic Ocean only connected by a narrow channel and with a semidiurnal tidal circulation (Lopes et al., 2007). This complex ecosystem is comprised by four main channels, two artificial jetties, small islands and mudflats (Queiroga et al., 2006). Ria de Aveiro is considered the most important humid zone in northern Portugal and is listed as a Special Protection Area (SPA) being part of the Natura 2000 network (ICNF). The protected zone includes a wide variety of biotopes such as seagrass beds, mudflats, reed beds and the biggest saltmarshes area of Portugal (Lillebø et al., 2011). Its ecological importance is enhanced with the diversity of species groups that use the estuary as a nursery, feeding area or permanent habitat (Pombo et al., 2007; Lillebø et al., 2011; Rodrigues et al., 2011).

1.2.2. Economic importance

Estuaries provide a significant productivity on a global scale. The most productive species found in Ria de Aveiro includes fishes (i.e. sardine and pout), bivalves (i.e. oysters, common cockle and grooved carpet shell), molluscs (i.e. cuttlefish and octopuses), crabs (i.e. green crab), worms (used mostly as fishing baits) and macro-algae all sharing a relevant commercial value (Lillebø et al., 2011).

Several other sectors, besides fisheries, are also intrinsically connected to the estuary such as aquaculture, salt production, industry, agriculture and tourism (Lopes et al., 2007; Lillebø et al., 2011).

Nevertheless, the continued and growing usage of this area brings some concerns when it comes to quality and integrity of the natural system (Klassen and Locke, 2007; Pombo et al., 2007).

1.3. The species in study: *Carcinus maenas*

The species *C. maenas* belongs to Order Decapoda (suborder: Brachyura) Family Portunidae. It is commonly termed as European green crab, green crab and shore crab, among others, depending on the speaking language (Yamada, 2001). It is one of the most well-studied marine species, having a relevant ecological importance in estuarine and inter tidal environments (Queiroga, 1996; Yamada, 2001; Moksnes, 2002; Beata et al., 2005; Amaral et al., 2009).

Its natural distribution includes the coastal and estuarine waters of Europe and Northern Africa; however, this species is also a successful global invader that prospers in other parts of the world, such as both coasts of North America, South Africa, Australia, South America, and Asia (Yamada, 2001; Klassen and Locke, 2007).

In Portugal the green crab can be found along the coast of its west extension with well-established populations, being the most common crab in estuarine ecosystems.

This brachyuran crab exhibits a complex biphasic life cycle, a common feature for several marine invertebrates, with 4 pelagic zoeal stages and a post-zoeal form called megalopa (Figure 1), developing and dispersing in the water column (Yamada, 2001; Calado and Leal, 2015). The free-swimming larvae may take 18 to 48 days to reach metamorphosis (Beata et al., 2005), an event considered as a very important and dramatic transition from pelagic to benthic life (Beata et al., 2005; Calado and Leal, 2015). Each development stage ends up with a moulting process, whose rate decreases once the species attains adult size (Yamada, 2001).

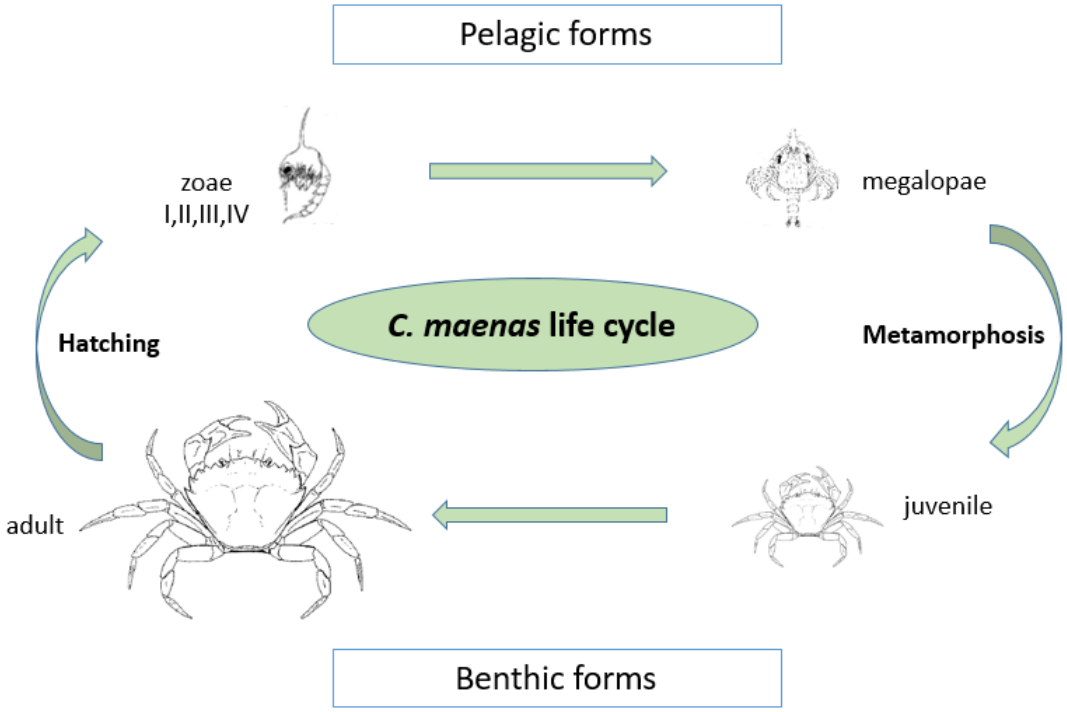


Fig.1: *Carcinus maenas* life cycle.

Concerning its morphology, *C. maenas* stands out among other species in family Portunidae by displaying the fifth leg clearly adapted for walking instead of swimming (Yamada, 2001). The adult body width varies approximately from 70 to 90 mm for females and males, respectively. The lateral margins of the carapace present five spines/teeth equally spaced and sized. Its most common coloration is dark olive-green but it can also display other tones such as red, yellow and orange (Yamada, 2001; Klassen and Locke, 2007).

C. maenas can survive at temperatures ranging from 0 to 35 ºC with a high salinity tolerance (from 4 to 52), explaining the status it yields as a global invader (Yamada, 2001; Almeida et al., 2011). Previous studies have shown that *C. maenas* inhabits a wide variety of habitats having preferences, especially juveniles, for more complex subtracts like seagrass beds, mussel beds and rocky intertidal, providing better protection from predators and older conspecifics (Hedvall et al., 1998; Moksnes, 2002; Almeida et al., 2008).

While in the water column, green crab larvae scoop up plankton (Yamada 2001). Their diet mostly includes planktonic particles in the size range of bacteria, micro- and meso- zooplankton, small algal cells and organically enriched detrital

particles (Factor and Dexter, 1993; Coelho et al., 2009). Accordingly to Coelho et al. (2009), *C. maenas* larvae also ingests phytoplankton when other food sources are not available avoiding the possibility of larvae starvation. Later on, at a juvenile crab form, detritus are replaced by bigger organisms as those found in the infauna (Yamada, 2001; Klassen and Locke, 2007).

As an adult, *C. maenas* is known to be a voracious omnivore and an opportunistic predator (Yamada, 2001; Almeida et al., 2011) ingesting a wide variety of dietary items (Cohen and Carlton, 1995; Klassen and Locke, 2007). Its diet is mainly composed by molluscs, crustaceans, and polychaetes (Beata et al., 2006), although in some studies it is believed to differ accordingly to several factors such as geographic differences, prey availability, crab size, colour and sex (Cohen et al., 1995; Beata et al., 2006). Cannibalism is also known to be usual among adults (Almeida et al., 2011), contributing to the shaping and regulation of the populations (Lovrich et al., 1997).

In terms of ecological and economic relevance, *C. maenas* may cause great damage to areas where it appears as an invasive species. The negative impacts on local species can be caused by an increase of predation, competition for food and habitat (Beata et al., 2006). *Carcinus maenas* presence may not only influence local species but also modify marine ecosystems. In the United States, for example, several studies reveal that the production of some bivalve species are at risk, as well as other crab species, since *C. maenas* reveals a strong territorial behaviour and predation in juvenile stages of these organisms (Jamieson et al., 1998; Klassen and Locke, 2007). Marine vertebrate species are also affected representing losses to commercial fisheries higher than $44 million per year (Jamieson et al., 1998; McDonald et al., 2001; Grosholz et al., 2002).

Carcinus maenas is presently used by researchers as a model species to better understand the biology and ecology of marine invertebrates with a biphasic life cycle. A better understanding of its behaviour represents an important information not only for this particular specie but for the marine environment.

1.4. Objectives

Canal de Mira, Ria de Aveiro, function as a nursery area for the specie *C. maenas*, having several peaks of megalopae entering the estuary along the year to settle and develop (Queiroga et al., 2006). After metamorphosis, the young crabs may stay close to the settlement area for several weeks, increasing the possibilities of contact with conspecific (Moksnes, 2004b; Almeida et al., 2011). As such, interactions like cannibalism are most likely to occur.

The aim of this study is to understand if cannibalism can occur during settlement events between megalopae, as well as when these are in contact with the first three crab stages of *C. maenas*. Under laboratory conditions, we tested the influence of density, habitat and food as frequent triggers responsible for this behaviour on marine environments. Therefore, our null hypotheses were as follows: (1) density, habitat complexity and food availability do not trigger cannibalism among megalopae; (2) There is no cannibalistic interaction between fed/unfed crabs at C1 stage and megalopae after settlement; (3) There is no cannibalistic interaction between the first crab instars C1, C2 and C3 towards megalopae.

2. Materials and Methods

2.1. Study site and collection of wild megalopae

Canal de Mira is one of the 4 main channels of Ria de Aveiro (Lillebø et al., 2011); it presents excellent conditions for *C. maenas* to settle and develop, displaying high food availability and different types of complex habitats (Queiroga et al., 2006).

Wild megalopae were collected at Costa Nova (Canal de Mira, 40° 37' 17 N, 8° 44' 56" W) during four consecutive supply events (see below). For each event we used two plankton nets (as described by Queiroga et al., 2006) which were placed for 24 h, at a constant depth of 0.1 m, with their entrances facing the inlet of the estuary. Plankton kept in the collector cup was gently rinsed and poured to a bucket filled with seawater and transported to the laboratory for analysis. Larval collection was carried out during five consecutive days every full/new moon between March and May of 2015.

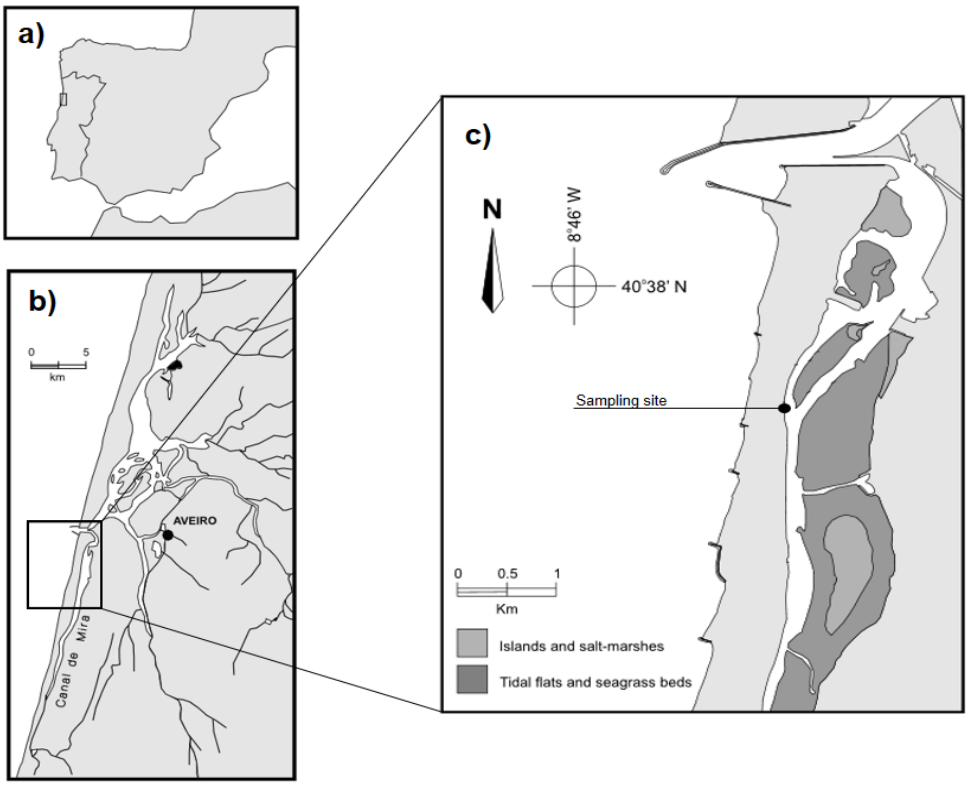


Fig.2. The study area. a) Location of the estuary at the Portuguese coast. b) Ria de Aveiro. c) Sampling location were the plankton nets were placed (Adapted from Queiroga et al., 2006).

2.2. Experimental setup

During each supply event, megalopae were individually picked from the plankton sample and collected in the laboratory using a light table and a modified plastic pipette.

All experiments were performed in Petri diches (with a volume of 23 mL) filled with artificial seawater (prepared by mixing Tropic Marin ProReef salt with purified freshwater from a reverse osmosis unit). Controlled conditions of temperature (20 ºC), salinity (35) and photoperiod (12:12 h dark:light) were applied for all the treatments. For those where individuals were fed, we provided newly hatched *Artemia* nauplii at a density of 3 nauplli /mL and used shells of blue mussels (*Mytilus galloprovincialis*) as habitat (one shell per individual with approximately 15mm high and 9mm length).

To test the survival of megalopae under the experimental conditions, the experimental procedures were divided into three groups (Experiment 1, 2 and 3) accordingly to the exposure of megalopae with different *C. maenas'* life stages with every treatment lasting for 24 h (see Fig. 1). Experiment 1 (N=320 for all treatments with 2 megalopae and N=640 for all treatments with 4 megalopae) was performed only with megalopae, stocked at two different densities (2 and 4 megalopae/Petri dish approximately 870 and 1739 megalopae/m^2), both with and without shelter and with and without food. At experiment 2 (N=280), two sets of C1 juveniles, raised under laboratory conditions from megalopae collected from the wild (as described above), were used as predators -one set originated from fed megalopae that metamorphosed to C1 (C1_mf) and the other originated from unfed megalopae that also metamorphosed to C1 (C1_mu). Once again habitat complexity (with and without shelter) was used for both sets of predators. The individuals were not feed throughout this experiment. In experiment 3 (N=160) we used sets of C2 and C3 juveniles, originated from megalopae collected from the wild (as described above), as potential predators for megalopae. Food and habitat were applied as described at experiment 2. A control test (1 megalopae/Petri dish approximately 435 megalopae/m^2) was also conducted throughout all the experiments to ensure that megalopae mortality was only due

to cannibalism and no other factors. For all experiments, cannibalism was always directed towards the megalopae and not otherwise.

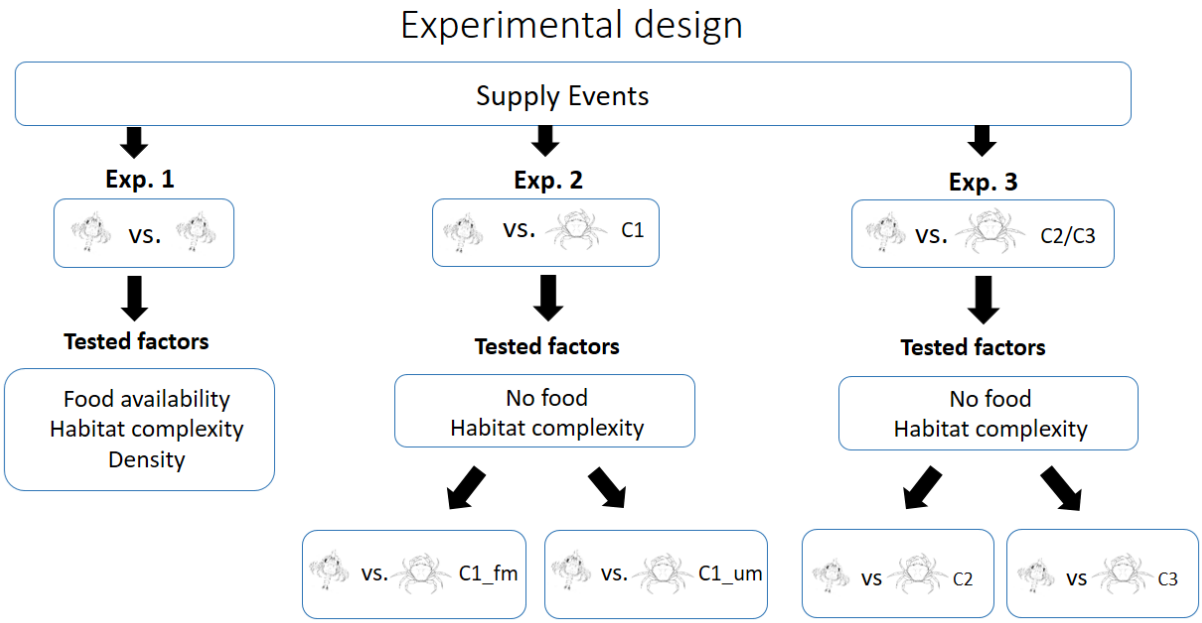


Fig.3. Experimental design of all treatments. Experiment 1: megalopae vs megalopae at different densities; Experiment 2: megalopae vs Crab1 originating from fed megalopae (C1_fm) and from unfed megalopae (C1_um); Experiment 3: megalopae vs Crab2 / Crab3.

2.3. Statistical analysis

Multivariate statistical analyses were performed to detect the existence of significant differences in survival of megalopae, and understand which factors could promote cannibalistic behaviours between them (Mertler and Vannatta, 2002 pag. 15). To conduct the Permutational MANOVA, density (D), habitat (H) and food (F) were used as fixed factors. Prior to the statistical analysis the raw data matrix was log (x +1) transformed and a new matrix was assembled using Bray-Curtis similarity coefficient. All multivariate statistical tests were performed on Primer 6.1 with PERMANOVA add-on (Primer-E Ltd. Plymouth, UK).

Two-way contingency tables were applied for experiences 2 and 3, testing the independency of the variables, followed by Pearson's Chi-squared test and Fisher's exact test respectively (Yates, 1984; Agresti, 2002). Megalopae survival

was used as the frequency distribution, crossing the categorical variables habitat and predator.

3. Results

3.1. Experiment 1: Density-dependent cannibalism between megalopae

The analysis of megalopae survival revealed the existence of significant statistical differences promoted by density, with p-value = 0,0437. Cannibalism between megalopae increased at higher densities (Fig. 4). Food availability and habitat complexity had no significant effect on megalopae survival, with p = 1 and p = 0,3178 respectively.

Fig.4. Megalopae survival (%) accordingly to each treatment - nF= No Food, nH= No Habitat, wF= with Food, wH= with Habitat.

3.2. Experiment 2: Feeding regimes and habitat complexity

A Pearson's Chi-squared test was used for the experiment analysis resulting on the acceptance of the null hypotheses (p = 0,9347). Megalopae survival rates were a little higher when in contact with C1_fm presenting a survival rate of 95% and 93,33% with and without shelter respectively, dropping to 90% and 91,25% (with and without shelter) when in contact with C1_um (Fig. 5). However, the results showed no significant associations between the factors habitat and crab concerning the survival of megalopae. The association plot showed that megalopae survival was lower than expected when put together with C1_um without habitat and higher than expected on C1_um with habitat. Opposite results were observed with C1_fm. (Fig. 6.a). All factors of this experiment are near to complete independency, as shown in the mosaic plot (Fig. 6.b).

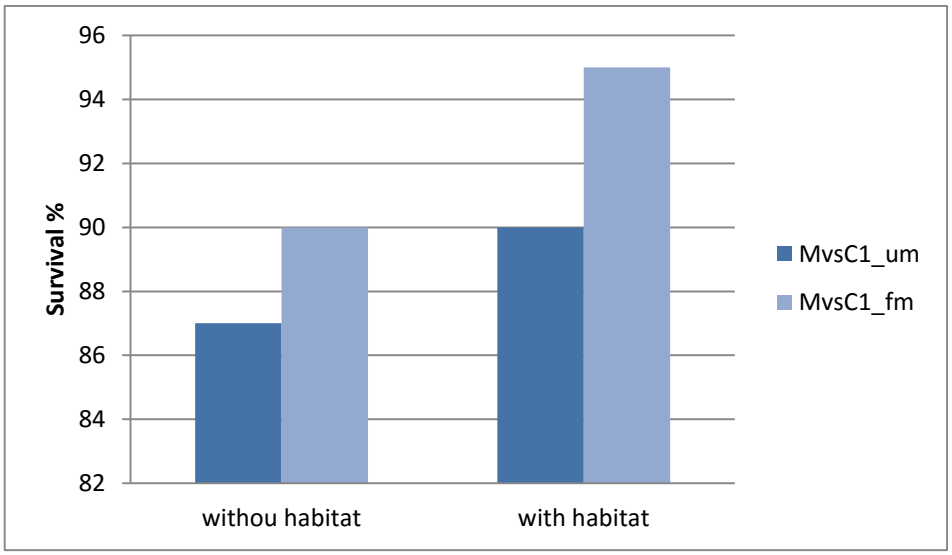
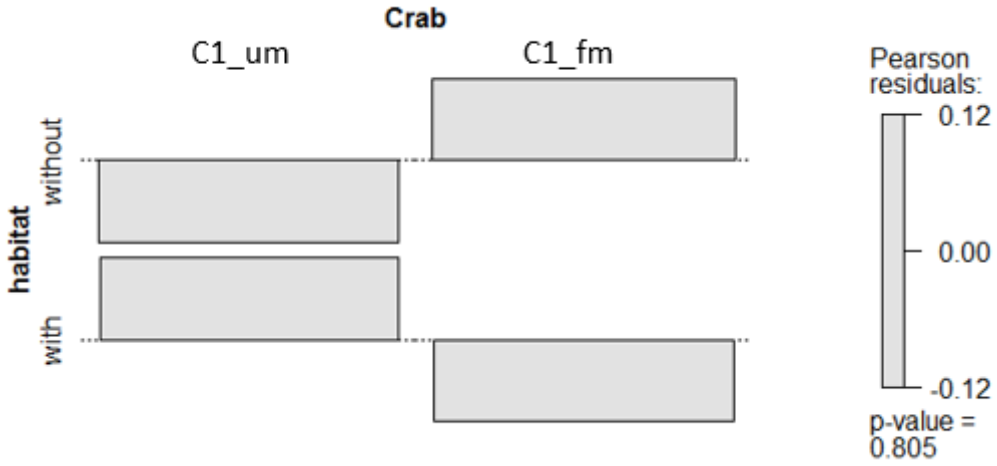


Fig.5. Megalopae survival with C1 from unfed and fed megalopae as potential predators, with and without habitat.

a)



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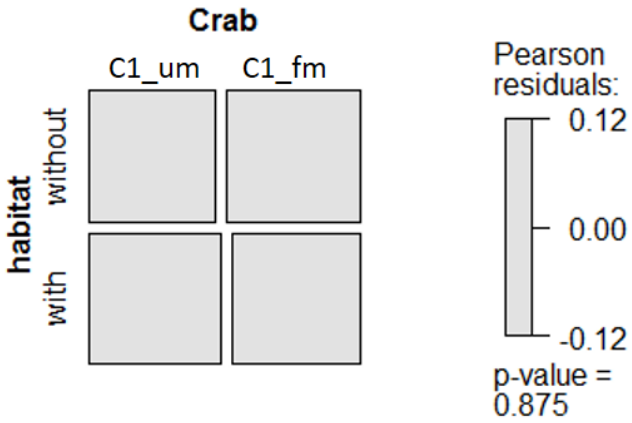


Fig.6. Pearson's Chi-squared test for significance measure (p=0,9347) between C1 from fed and unfed megalopae and habitat complexity: a) association plot with expected frequencies; b) mosaic plot for independence. The grey area represents the survival of *C. maenas* megalopae after 24h in the presence of different conspecific life stages.

3.3. *Experiment 3*: Cannibalism and different crab stages

The null hypothesis was rejected as well on experiment 3, with no significant differences being recorded on megalopae survival between crab stages and habitat (p=1). Nevertheless, megalopae survival rates decreased expressively when in contact with C2 and C3 juveniles with 77,5% and 60% (with and without shelter) and 17,5% and 10% (with and without shelter) respectively (Fig.7). Moreover, there is a tendency at the expected frequencies to show higher differences of the ones observed, as the crab stages advance (Fig. 8.a). The

association observed at the mosaic plot (Fig. 8.b) shows that megalopae's survival decreases significantly on the presence of C3 crabs when compared to C1 and C2. Cannibalistic interactions increased with later crab stages.

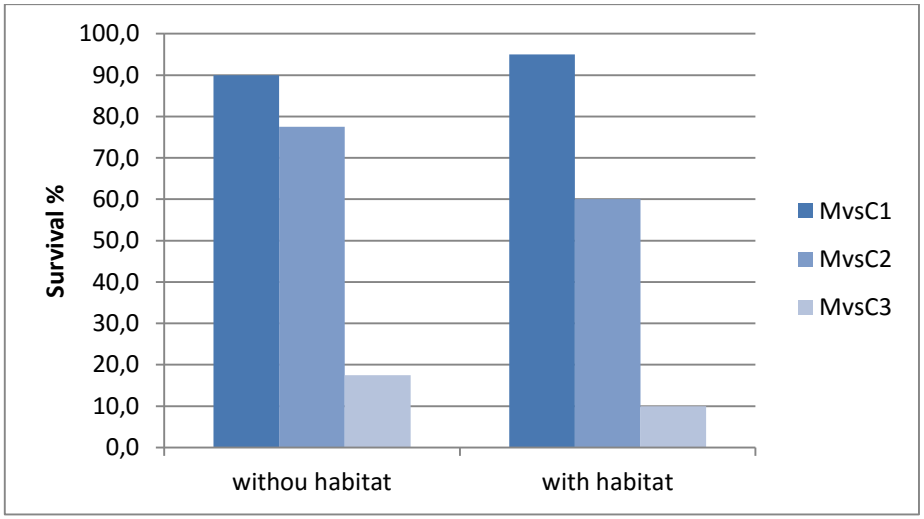
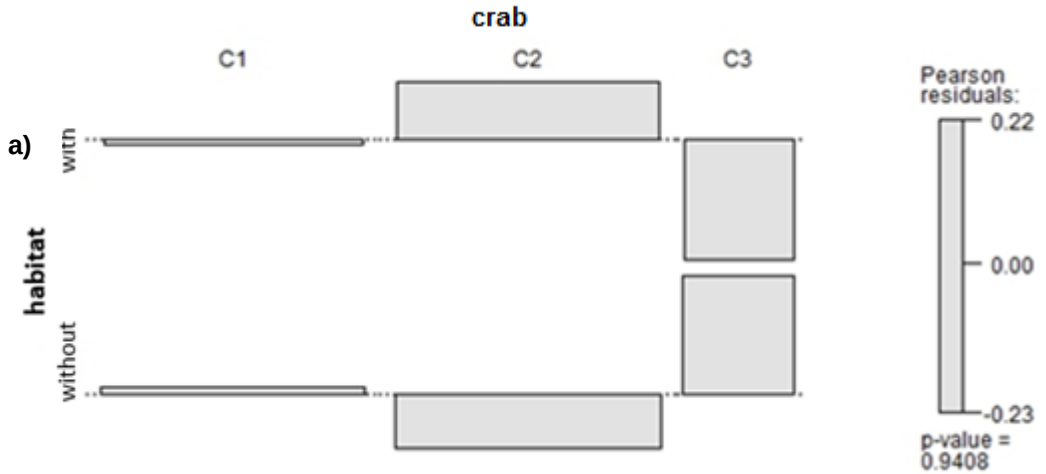


Fig.7. Megalopae survival with C1, C2 and C3 juveniles as potential predators, with and without habitat.



b)

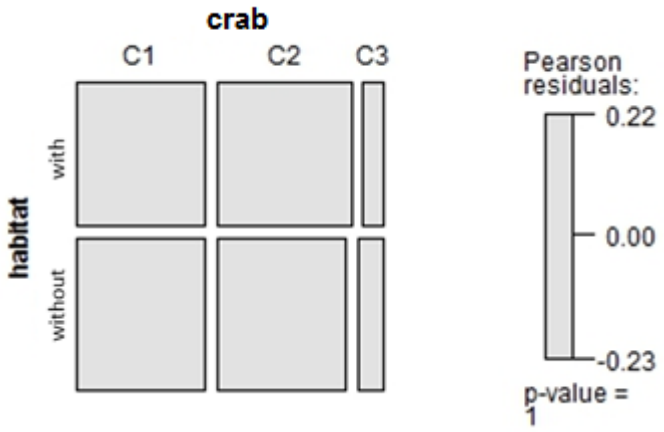


Fig.8. Fisher's exact test (p=1) between different crab stages and habitat availability **a)** expected frequencies; **b)** mosaic plot. The grey area represents the survival of *C. maenas* megalopae after 24 h in the presence of different conspecific life stages.

4. Discussion

4.1. Density-dependent cannibalism between megalopae

Megalopae's mortality promoted by cannibalism was not affected by habitat complexity and food availability but changed significantly between densities. All treatments with 2 megalopae presented a survival rate of 100% dropping to 99% on treatments with 4 megalopae. These results suggest that cannibalistic tendencies between megalopae of *C. maenas* are primarily affected by the increase of megalopae densities during settlement events.

Cannibalistic behaviours were also verified between megalopae of other crab species like the mud crab, *Scylla serrata,* (Rabbani and Zeng, 2005; Webley et al., 2009) and the king crab (from the Lithodidae family), *Lithodes santolla* (Sotelano et al., 2012). Moksnes (1997) observed intra-cohort cannibalism between the blue crab megalopae but concluded that mortality was much less than the one observed between cohorts and under circumstances of high megalopae densities at noncomplex habitats. Ventura et al. (2008) also tested cannibalism between megalopae and zoae larvae on *Ucides cordatus* on a larviculture regime and found that megalopae do attack particular zoeal stages.

Nevertheless, some species are known to be less aggressive than others. For example, the mangrove crab *Ucides cordatus* showed 100% of megalopae survival independently of the conditions provided (Ventura et al., 2008).

Even though cannibalism was only observed at the highest density (1739 meg/m2) this value exceeds the one found under natural conditions at Ria de Aveiro (Queiroga et al., 1994; Queiroga, 1998). One possibility is that the main priority to megalopae is to find a suitable habitat to metamorphose (Moksnes, 1997; Hedvall et al., 1998; Almeida et al., 2011) and even though the possibility for contact between conspecifics at the same life stage does exist during settlement events, cannibalism may only be expected to occur at abnormally high densities. According to Moksnes (1997, 1998) one of the principal causes of blue crab (*Callinects sapidus*) megalopae mortality during settlement is the presence of predators and older conspecifics, not conspecifics at the same life stage (other megalopae). Similar results were also obtained in Moksnes (2002) for *C. maenas*.

4.2. Feeding regimes and habitat complexity

Cannibalism between megalopae and first instar juveniles was not frequent, even when C1 originated from megalopae that were not fed. On previous studies (Giménez, 2004; Rey et al., 2015), pre-settlement factors such as megalopae feeding regimes promoted latter effects on the size and energy reserves of juvenile crabs. In our results food availability during megalopae development into C1 did not influence cannibalism towards megalopae. As such, other factors could also be considered. For example, as observed on experiment 1, density can also be applied in this scenario. Even though the probability of contact between megalopae and C1 was high at the lowest density, the presence of other conspecifics at same stage could enhance the aggressiveness of C1 towards megalopae.

Concerning the presence or absence of habitat, it had no impact on the behaviour of megalopae and juveniles. These results contrast with those reported by Luppi et al. (2001) for *Chasmagnathus granulata* and *Cyrtograpsus angulatus*, where habitat provided shelter for juveniles against adult conspecifics' predation but not against other juveniles that easily had access to the same shelters. In our

experience, the absence of agonistic behaviour of C1 towards megalopae could somehow be justified by the small size differences that they presented, which could somehow inhibit cannibalistic behaviours.

Under natural conditions, choosing the most adequate habitat as a post metamorphosis organism is an important step for survival and development (Moksnes et al., 1998; Luppi et al., 2001; Moksnes, 2002). Moksnes and Heck (2006) confirmed that megalopae of the blue crab did not seek for specific complex habitats to settle but the first structured one that they could reach. Later on, C1 juveniles have the ability to move efficiently and look for a better place to hide right after metamorphosis. Similar results were observed by Moksnes (2002) with *C. maenas* megalopae. In both studies the occurrence of cannibalistic behaviours was recorded from juvenile crabs towards megalopae. However, other factors that were not used in our experiment were also implied such as more complex shelters and the presence of conspecifics under the pressure of other predators, which also represented a major role on cannibalism.

4.3. Cannibalism and different predator stages

Direct observations showed us that megalopae were cannibalized immediately after being placed together with C3 juveniles. Comparing to first and second juvenile instar, C3 crabs presented the highest aggressive behaviour towards megalopae. This result is in line with several studies where encounters promoted by higher densities, same habitat choices and size differences between juveniles from different cohorts affects negatively on the younger juveniles' survival (Moksnes, 2004a, b). Knowing that our experiment simulated worst case scenarios (no food and no shelter), we also believe that this results are consistent with those reported by Moksnes et al. (1998) where older juveniles with a particular size difference reacted to smaller ones as if they were a regular prey.

Cannibalism is a constant behaviour among crustaceans and it is believed that is more representative and common among cohorts from different years (Moksnes, 1997; Sotelano et al, 2012). The variety of sizes, densities and predator pressures among cohorts makes cannibalism a consequent reaction with a high impact on population self-regulation. In this study similar pressures were also

verified among cohorts from consecutive events. The presence of juvenile at different stages (for example C3 juveniles) from earlier settlements, may represent a significant regulation trough cannibalism on larvae supply for the population.

Concluding, it can be predicted that even when megalopae are not predated after settling peaks, their abundance is controlled by other conspecific through cannibalistic interactions. It is also possible that under scenarios of food depletion higher densities of settlers can become an alternative food source (Luppi et al., 2011).

5. Final considerations

With the present study we verified that adult populations of brachiuran crabs, found on estuarine areas such as ria de aveiro, might not represent the full potential presented by consecutive settlement events. The presence of juveniles from early settlement cohorts affects the larvae survival trough cannibalism independently of food regimes and shelter availability. This results complements those from Moksnes (2002, 2004a and 2004b) on *C. maenas,* frequently referred as one of the best models for marine invertebrate species with a complex lifecycle.

As all experiments were conducted under laboratory, although mimicking as closely as possible the scenarios observed under natural conditions, our results should be interpreted with conditions. The study of small sized marine organism such as larvae still represents a remarkable challenge to be accomplished on the wild. In this way, a simplification of these highly complex ecosystems is often necessary to shed some light over key processes of their life cycle, such as the transition from the pelagic environment to early benthic life. Nevertheless, our results allow us state that cannibalism does occur during settlement events of *C. maenas*, even under favourable conditions of food availability and refuge. As such it confirms its importance on intraspecific regulation establishing a natural limit on the population abundance (Lovrich and Saint-Marie, 1997; Moksnes, 2004b; Almeida et al., 2011). In an area of high ecological and economical importance such as Ria de Aveiro where *C. maenas* is well established, this self-limitation might also be a control on predator impacts caused on the other native species.

We personally observed as some megalopae shortly after being in contact with C1 went through metamorphosis. Nonetheless, although being in highly vulnerable stage, they were never predated by C1 conspecifics. The size similarity and low density might be the explanation for the absence of cannibalism between these two consecutive life stages, as already highlighted by Luppi et al. (2001). Other studies (Dutil et al., 1997; Lovrich and Sainte-Marie, 1997; Stevens and Swiney, 2005; Zmora et al., 2005) also explained the relevance of size differences within and between cohorts for cannibalistic interactions. Hedvall et

al. (1998) reported that megalopae of *C. maenas* are still predated by C7 and C8 conspecific crabs. It would be interesting to test if more advanced instars, i.e. C9 and C10, still predate on megalopae and if so it could certainly be interesting to model the impact of this behaviour on the shaping of adult populations.

Carcinus maenas has a particular tolerance for a wide temperature range and it is known that this environmental factor affects its activity (Ropes, 1968; Taylor et al., 1977; Moullac and Haffner, 2000), as well as the majority of other crustaceans (Hill, 1980; Pavasovic et al., 2004; Stoner et al., 2010). Testing which temperatures could enhance or inhibit cannibalistic behaviours of C3 and older juveniles would be a good follow-up study on intraspecific interactions studies, with emphasis on cannibalism, taking into account the scenarios currently predicted for ocean warming.

We believe that there is more to know about cannibalism in marine invertebrate species and more studies are necessary to allow a better understanding on how this extreme intraspecific interaction can control and shape adult populations.

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Annex I

Detailed Description of the experiment

Fieldwork:

All sampling was scheduled to be held in the days of full moon and new moon. At these particular moments the number of successful catches are greater matching settlement peaks and megalopae's entrance in the estuary channel.

In the days prior to each event all field and laboratory equipment was assembled and separated. Sampling was always initiated in the morning at the sailing school of Costa Nova in Canal de Mira. We used two plankton nets (Figure II 4) which were placed on the farthest pontoon of the marina. About 30 m away from each other, the plankton nets were left in the water for 24 h. After that time, we returned to the site to collect the contents retained in the larval collector (Figure II 3) of each net. The nets were once again placed in water and all the process was repeated on the next morning.

Before starting the sampling, it was necessary to gather a small amount of mussels (*Mytilus galloprovincialis*) to use their shells as habitat in the experimental trials. The best way to separate the shell of the mollusc was by putting them in boiling water for a few minutes and remove the organic parts individually.

Laboratory:

Plastic buckets were always used for the magalopae's transport to the laboratory where they were then separated from the remaining organic material also retained in the larval collector. For this process, the content of the buckets was divided by rectangular plastic trays, where the megalopae were collected individually with the aid of plastic pipettes. After counting the megalopae, they were distributed to Petri dishes according to the experiments (II Figures 5 and 6) or maintained individually in PVC containers (II Figure 7) so we could raise them into juvenile crabs (II Figure 8).

The room where the experiments took place was acclimatized and maintained at a constant temperature of 20 ° C.

All experiments had a duration of 24 h after which we registered the results.

At the end of each experiment the megalopae and juveniles were collected, fed and kept in proper environment to be returned to the Ria the next day. All material used was thoroughly washed in running water and replaced to the following experiments.

Raising the juveniles:

In order to know for certain juvenile crab stages, they were fed ad libitum with *Artemia* nauplii and monitored every day. The water was also changed with the same frequency after washing the PVC trays. We determine the crab stages every time we collected an exuviae from the containers, always recording the number of days that juveniles remained in each stage, as well as the number of dead specimens.

Annex II

Photos of the fieldwork and laboratory experiments

Figure II 1- Assembling the nets in the pontoon of the “Escola de Vela da Costa Nova”.

Figure II 2- Placing the nets in the water. Researcher: Gina Neto.

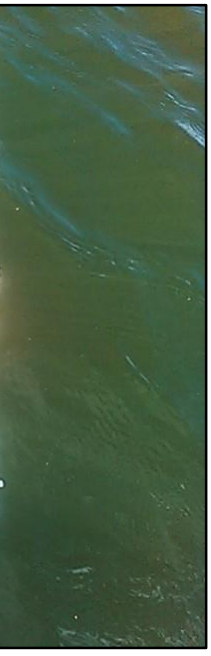

Figure II 3- Overview of the larval

Figure II 4- Overview of a

Figure II 5- Experiments with 2 and 4 megalopae with habitat.

Figure II 6- Experiment C1 (a) with one megalopae (b).

Figure II 7- Juvenile C3 holding a megalopae.

Figure II 8- Juvenile C3 feeding on a megalopae.

Figure II 9- Juvenile culture on PVCs container.

Figure II 10- *Carcinus maenas* juvenile.